The Amount of Methyl Parathion Accepted by Larvae of the Almond Moth, Cadra cautella Walker, in Dry Film Method

AUTHOR(S):

HASHIMOTO, Yasushi; GOTO, Shinko

原　著

The Amount of Methyl Parathion Accepted by Larvae of the Almond Moth, *Cadra cautella* Walker, in Dry Film Method. Yasushi Hashimoto and Shinko Goto (Agri cultural Chemicals Inspection Station, Ministry of Agriculture and Forestry, Kodaira, Tokyo) Received October 15, 1968. *Botyu-Kagaku* 34, 1, 1969.

1. Dry film 法におけるコナマダラメイガ幼虫のメチルパラチオン附着量 橋本　康・後藤真康（農林省農薬検査所，小平市，東京都）43. 10. 15 受理.

殺虫試験を dry film 法で行なった場合，供試昆虫はシャーレに処理された農薬をどの程度虫体に附着させるのか検討した．シャーレにメチルパラチオンの dry film を作り，これにコナマダラメイガ幼虫を接触させたところ，24時間後にはシャーレ中のメチルパラチオンは処理量の約 50% になり，約 50% が虫体に附着したと推定された．また同時に topical application を行なった．その結果，1個体あたりの処理薬量が同じであれば，いずれの方法でも大体同じ致死効果の得られることが認められた．

"Dry film method" or "dry residue method" is one of the most popular methods for bioassay of insecticides. As this method is handy, it has been widely applied in screening test or evaluation of the resistance level of insects. But the accurate amount accepted by individual insect is very difficult to determine by this method.

Thus, an attempt was made to presume the amount of an insecticide accepted in insects by analyzing residue of the insecticide in the dry film. The presumed amount of the insecticide was almost equivalent to the lethal dose applied by topical method.

Material and Method

Insect: The last instar lervae of almond moth, *Cadra cautella* Walker were used. They are from the methy-parathion resistant strain developed in a rearing room of Agricultural Chemicals Inspection Station.[1)]

Dry film method: Methyl parathion was dissolved in acetone so as to give desired doses in 1 m*l* of solution. The solution was pippeted into a petri-dish of 9 cm in diameter to make uniform dry film on the bottom. Then 20 male insects were kept in each dish for 24 hours. After contact, the insects were removed and the dishes were subjected to analysis for methyl parathion. Blank dishes only with methyl parathion and only with insects were prepared concurrently and analyzed in the same way as the treated dishes.

Topical application: Acetone solutions of methyl parathion were prepared so as to give desired doses in 8 μl of the solutions, which were applied onto dorsal part of insects by a micrometersyringe. Twenty male insects in each dose were treated and then kept in a dish for 24 hours to observe mortality.

Analytical method of methyl parathion in dish: The colorimetry based on the Ketlaar's method[2)] was applied. The inside surface of each dish was washed repeatedly with 1N potasium hydroxide-methanol solution. The washing was pooled in a 25 m*l* measuring flask. After a few drops of 30% hydrogen peroxide solution was added, it was allowed to stand overnight. Then the reacted mixture was diluted with methanol to 25 m*l* and the absorption density was measured at 400 mμ. Amount of methyl parathion was calcurated by a calibration curve obtained from pure substance of *p*-nitrophenol. Since methyl paraoxon, *s*-methyl isomer of methyl parathion and *p*-nitrophenol are measured as methyl parathion by this method, thinlayer chromatography was also applied to confirm methyl-parathion. Recovery of methyl parathion by this method was being 95～105%.

Calcuration of accepted amount by insect: Presumed amount of methyl parathion accepted by insects in each dish was calculated by the following equation:

$$D = T - (R - C)$$

where D = accepted amount by insects
T = initial dose in dish
R = recovered amount in dish after insects

were removed

C=correction factor

The above-mentined analysis was also carried out on the blank dish only with insects, some substances equivalent to 1.8μg of methyl parathion were detected. These substances seem to attributable to the feces and other excreta of test insects. Then the correction factor C was added to the analytical result in each dish. However, no correction was made to allow for error due to recovery.

Results and Discussion

Experiments on dry film method were repeated three times and the results were averaged. Presumed amount accepted by insects, its percentage to initial dose in the dish and the mortality of insects are shown in Table 1.

The experimental results show that larvae of the almond moth accept methyl parathion in proportion to the initial dose in the dish: they accept about 50% of the insecticide deposited in the dish irrespective of initial doses.

Results of topical application are given in Table 2. They are the average of two series of experiment. The mortalities in dry film method and topical application were plotted as shown in Figure 1. This figure shows that if the initial dose per insect was same, almost same mortality was obtained without regard to the application methods. Thus, the topical application was recognized to be reasonably substituted by the dry film method.

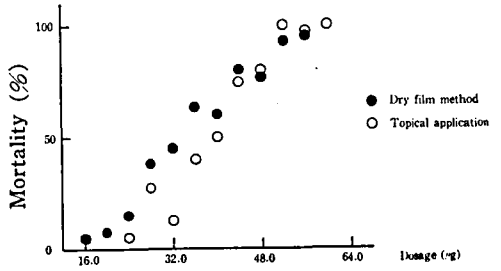

Fig. 1. Relationship between the mortality of the almond moth larvae and dosage of methyl parathion treated by the dry film method and topical method.

Table 1. Comparison between initial dose of methyl parathion in a petri-dish, accepted amount by larvae of the almond moth and insect mortality.

Initial dose (μg)	8.0	9.4	11.2	13.4	16.0	18.8	22.4	26.8	32.0	37.6	44.8
Accepted amount (μg)	4.0	5.2	4.2	5.9	8.7	6.7	9.2	11.4	13.9	14.0	17.9
Percentage accepted (%)	50.0	55.3	37.5	44.0	54.0	35.6	41.1	42.5	43.4	37.2	40.0
Mortality (%)	5.0	6.7	15.0	38.3	45.0	63.3	60.0	80.0	76.7	93.3	95.0

Table 2. Results of topical application of methyl parathion to larvae of the almond moth.

Dose per insect (μg)	0.47	0.56	0.67	0.80	0.94	1.12	1.34	1.60	1.88	2.24	2.68
Dose per 20 insects (μg)	19.4	11.2	13.4	16.0	18.8	22.4	26.8	32.0	37.6	44.8	53.0
Mortality (%)	0	5.0	27.5	12.5	40.0	50.0	75.0	80.0	100	97.5	100

Summary

Experiments were undertaken in order to find out the amount of pesticide accepted by test insects by dry film method. Dry film of methyl parathion was formed in a petri-dish, to which larvae of the almond moth were exposed. After 24 hour exposure the larvae were removed and methyl parathion remained in the dish was chemically analyzed. The amount of methyl parathion in the dish was found to be about 50% to the initial dose, and remaining 50% was considered to be accepted by insects.

When methyl parathion was applied to the insects either the dry film method or the topical method, almost same mortality was obtained if the initial dose per insect was same.

Aknowledgement: The authors wish to express

their appreciation to Dr. T. Suzuki and Mr. A. Sugimoto, Agricultural Chemicals Inspection Station, for their valuable suggestions and criticisms.

Literatures Cited

1. Hashimoto, Y., and Fukami, J: *Jap. J. Appl. Ent. Zool.* 8, 62 (1964).
2. Ketelaar, H.: *Anal. Chem.* 23, 64 (1951).

Determination of Insecticide Residue in Animal and Plant Tissues. VI. Determination of Sumithion Residue in Cattle Tissues. Junshi Miyamoto and Yoshishige Sato (Agricultural Chemicals Research Department, Osaka Works, Sumitomo Chemical Co., Ltd. Osaka) Received December 27, 1968, *Botyu-Kagaku* 34, 3, 1969.

2. 動植物組織中における残留殺虫剤の定量. VI 牛の組織中におけるスミチオン残留量の測定

宮本純之・佐藤香重（住友化学工業株式会社 大阪製造所 農薬研究部） 42. 12. 27 受理.

牧草の害虫防除に用いられたスミチオンの牛体内における残留をしらべるため，125 g/ha（通常の使用量）および 375 g/ha の割合でスミチオンを牧草地に散布し，その直後に放牧した牛の筋肉および脂肪組織中のスミチオンを経時的に定量した．

散布後24時間では，筋肉中に約 0.01ppm のスミチオンが残留するが，72時間以降では，両散布区とも筋肉中のスミチオン含量は 0.001 ppm もしくはそれ以下である．また高濃度散布区に放牧した牛の脂肪組織中には 72 時間後でごくわずかのスミチオンが見出されたが，7 日以後では，その量はほとんど無視できるくらいであった．

これらの結果からすれば，牧草とともに牛体内にとり入れられたスミチオンは，すみやかに分解されて消失し，その残留量は何ら問題とするに足りないと考えられる．

Introduction

Sumithion®, fenitrothion or *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate, is an organophosphorus insecticide characterized by the broad spectra as well as by low toxicity to warm-blooded animals and is now being widely used in the world to control harmful insects of various crops. This compound was found recently to be tremendously effective against locusts of pasture grass in Argentina and now it is put into practical use in the country. In order to assure safe usage of Sumithion from the view-point of public health, its residue in cattle tissues was determined after cattle had been kept on the pasture sprayed with Sumithion.

Materials and Methods

Sumithion spray and sampling[1]

Sumithion emulsion up to 125g/ha (usual application condition) and 375 g/ha in terms of the active ingredient was sprayed respectively to 5 ha (area A) and 8 ha (area B) of the test field in the suburbs of Buenos Aires, Argentina on July 23, 1968 and immediately thereafter each 10 head of cattle of about 300 kg in body weight were confined to the enclosures. One, three, seven and ten days after spraying, each 2 head of cattle were withdrawn from the area A and B and sent to a slaughter house. The next morning the animals were sacrificed, meat (breast muscle) and tallow (omental fat) dissected out and immediately transported to the laboratory.

Extraction of Sumithion and analysis.

i) Meat[2]

Fifty grams of minced meat was homogenized with 50 m*l* of water and 110 m*l* of ethanol, together with 5×10^{-6}g of ethylparathion as internal standard of gaschromatography, in a mixer for 30 sec., and then for further 1 min. after addition of 240 m*l* of benzene. The mixture was centrifuged at 2,500 rpm for 15 min. with an International Centrifuge, size 2, type V. The upper, benzene layer was separated and the lower layer as well as the precipitate was homogenized with 150 m*l* of benzene. Benzene layer was separated as above. Combined benzene layers were dehydrated over 100 g of anhydrous sodium sulfate overnight at room temperature. Sodium sulfate was separated by filtration, washed with 100 m*l* of benzene and